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PERSPECTIVES

CFTR channels and adenosine triphosphate release: the impossible rendez-vous revisited in skeletal muscle

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Adenosine triphosphate (ATP) crosses the cell membrane and modulates cell function of many animal and plant cells under various physiological situations. ATP is indeed considered as an autocrine/paracrine signal molecule in a variety of tissues. ATP release activates plasma membrane P2X and P2Y receptors to regulate tissue blood flow, ion transport, cell volume, and neuronal signalling. In epithelia, ATP acts as a potent secretagogue by increasing intracellular free calcium ion concentration thereby stimulating fluid and electrolyte secretion via binding purinergic receptors on the apical membrane. In vascular endothelial cells, ATP release is a well-known mechanosensitive response. ATP is thought to leave the cells after ionization to ATP^{4-} or $[MgATP]^{2-}$. Among the molecular candidates for ATP release pathways are ATP-binding cassette (ABC) proteins including P-glycoprotein and multidrug resistance protein (MRP), exocytosis of ATP-containing vesicles, pannexin and maybe ATP-permeable anion channels. However, despite these fundamental physiological roles and the description of numerous potential transporters, the molecular mechanisms underlying ATP release remain largely unknown.

The ABC transporter cystic fibrosis transmembrane conductance regulator (CFTR, ABCC7), whose mutation causes cystic fibrosis (CF), is an epithelial plasma membrane chloride (Cl^-) channel with transport activity under the tight control of intracellular cAMP and ATP concentrations. CFTR is a ligand-gated anion channel and a regulator of other membrane transporters. The physiological relationship between CFTR and ATP is complex and for some aspects still unclear. To translocate Cl^- across epithelial plasma cell membranes, CFTR uses the energy generated by ATP binding and hydrolysis at its two cytoplasmic nucleotide-binding domains named NBD1 and NBD2 (Riordan, 2008). Importantly, CFTR is gated by ATP, meaning that cytosolic hydrolysable nucleotides such as ATP and GTP are required to open phosphorylated CFTR. This is a unique dual mode of regulation with one or several ATP-dependent cycles of gating at the NBDs following R-domain phosphorylation by various protein kinases (Gadsby *et al.* 2006). It has also been suggested that CFTR functions as an ATP channel based on experiments comparing the ATP release of cells overexpressing P-gp or CFTR (Cantiello, 2001). ATP currents activated by cAMP were recorded in CFTR-expressing cells and evidence suggested that CFTR functions as a dual ATP and Cl^- channel (Reisin *et al.* 1994). However, a controversy appeared when it was shown that ATP was not conducted through CFTR in intact organs, polarized human lung cell lines, stably transfected mammalian cell lines, or planar lipid bilayers reconstituted with CFTR protein (Reddy *et al.* 1996). Furthermore, Grygorczyk & Hanrahan (1997) demonstrated that mechanical stimuli trigger CFTR-independent ATP release from epithelial cells. These findings rang the death knell of ATP permeation through CFTR and during the next 15 years this area of research was almost abandoned.

In light of several recent and novel findings, ATP permeation through CFTR might be resuscitated. First, recent studies enlarged the pattern of expression of CFTR in human cells by showing that smooth (Robert *et al.* 2005) and skeletal muscle (Divangahi *et al.* 2009; Lamhonwah *et al.* 2010; Tu *et al.* 2010) cells also express functional CFTR. Although the precise membrane location and physiological role of CFTR in these tissues are not known, some hypotheses have been formulated. In CF patients, exercise intolerance and skeletal muscle weakness are observed (Troosters *et al.* 2009) and since CFTR is expressed in muscle cells, it is proposed that CFTR controls Ca^{2+} homeostasis, muscular tone, metabolic recovery in exercise and diaphragmatic strength (Robert *et al.* 2005; Divangahi *et al.* 2009; Lamhonwah *et al.* 2010; Tu *et al.* 2010). Second, Cl^- transport due to CFTR stretch-mediated activation was described in the human airway epithelial cell line Calu-3. Stretch increased the open probability of single CFTR channels and stimulated CFTR-mediated anion secretion in Calu-3 cells and mouse intestine (Zhang *et al.* 2010). Third, in a study published in a recent issue of *The Journal of Physiology*, Tu *et al.* (2010) revisit the role of CFTR in ATP release.

Tu *et al.* (2010) investigated the effects of acidosis on ATP efflux from rat skeletal muscle. The expression of CFTR was confirmed using immunostaining in intact muscle and Western blotting in cultured skeletal muscle cells. Surprisingly, when the selective CFTR inhibitor $CFTR_{inh}172$ was applied to cultured skeletal myoblasts in the presence of lactic acid triggering the release of ATP, the acidosis-induced ATP efflux was abolished. Moreover, similar inhibition was recorded with a second, albeit less selective, inhibitor glibenclamide and after silencing the *CFTR* gene using RNA interference. Protein kinase inhibitor also blocked the acidosis-induced ATP efflux. But attempts to block mitochondrial ATP transporters using atractyloside failed to block ATP efflux. The authors therefore proposed that CFTR plays an important role in ATP release from rat skeletal muscle following depression of intracellular pH, a manoeuvre that enhances CFTR channel gating (Chen *et al.* 2009). This scheme for CFTR-dependent ATP release in skeletal muscle is clearly reminiscent of earlier results collected in cells expressing epithelial CFTR (Reisin *et al.* 1994; Cantiello, 2001).

Therefore, these results raise a number of very interesting and important questions for future studies regarding the role of CFTR in ATP release in mechanically dynamic environments. By studying CFTR-dependent ATP release from muscle, we should better understand whether ATP efflux is linked to the channel activity or to the regulatory function of CFTR. Equally important, could membrane stretch activate CFTR and ATP release in muscle cells? If CFTR mediates ATP release in muscle cells, is it triggered by mechanical stimuli? Future studies should be urgently conducted and solid experimental results acquired to address these questions. When these new pieces of the puzzle emerge, it

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should be possible to understand better the molecular mechanisms of ATP efflux in muscle and epithelial cells.

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